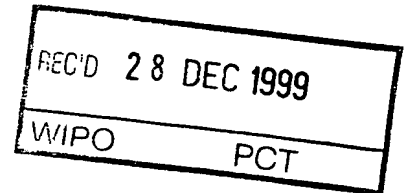


PRVPATENT- OCH REGISTRERINGSVERKET
Patentavdelningen

SE 99 / 1784

**Intyg
Certificate**

Härmed intygas att bifogade kopior överensstämmer med de handlingar som ursprungligen ingivits till Patent- och registreringsverket i nedannämnda ansökan.

This is to certify that the annexed is a true copy of the documents as originally filed with the Patent- and Registration Office in connection with the following patent application.



(71) Sökande *Karolinska Innovations AB, Stockholm SE*
Applicant (s)

(21) Patentansökningsnummer *9803393-9*
Patent application number

(86) Ingivningsdatum *1998-10-06*
Date of filing

Stockholm, 1999-12-17

För Patent- och registreringsverket
For the Patent- and Registration Office

A. Södervall

Anita Södervall

Avgift
Fee

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

**PATENT- OCH
REGISTRERINGSVERKET**
SWEDEN

Postadress/Adress
Box 5055
S-102 42 STOCKHOLM

Telefon/Phone
+46 8 782 25 00
Vx 08-782 25 00

Telex
17978
PATOREG S

Telefax
+46 8 666 02 86
08-666 02 86

A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY

Technical field

The present invention relates to novel molecules, such as proteins, polypeptides and nucleotides, involved in the hedgehog signalling pathway with putative involvement in embryonic development and carcinogenesis. The invention also relates to various novel advantageous uses of the molecules according to the invention in diagnosis and therapy.

Background

In the study of the development of cells, fruit flies have extensively been used as a model, as they are less complex than mammalian cells.

Pattern formation takes place through a series of logical steps, reiterated many times during the development of an organism. Viewed from a broader evolutionary perspective, across species, the same sort of reiterative pattern formations are seen. The central dogma of pattern formation has been described (Lawrence and Struhl, 1996). Three interlocking and overlapping steps are defined. Firstly, positional information in the form of morphogen gradients allocate cells into non-overlapping sets, each set founding a compartment. Secondly, each of these compartments acquire a genetic address, as a result of the function of active "selector" genes, that specify cell fate within a compartment and also instruct cells and their descendents how to communicate with cells in neighboring compartments. The third step involves interactions between cells in adjacent compartments, initiating new morphogen gradients, which directly organize the pattern.

Taking these steps in greater detail, one finds the first step in patterning to be the definition of sets of cells in each primordium. Cells are allocated according to their positions with respect to both dorsoventral and anterior/posterior axes by morphogen gradients. Allocation of cells in the dorsoventral axis constitutes the germ layers, such as mesoderm or neurectoderm.

In segmentation, the second step (the specification of cell fate in each compartment) is carried out by the gene *engrailed* and elements of the bithorax complex. *Engrailed* defines anterior and posterior compartments both in segmentation and in limb specification.

5

The third step in pattern formation, secretion of morphogens, functions to differentiate patterns within compartments (and thereby establish segment polarity). Initially, all cells within a compartment are equipotent, but they become diversified to

10

form pattern. Pattern formation depends on gradients of morphogens, gradients initiated along compartment boundaries. Such gradients are established by a short-range signal induced in all the cells of the compartment in which the above mentioned selector gene *engrailed* is active. For segment polarity, this signal is Hedgehog. In the adjacent compartment the selector gene is inactive, ensuring that the cells are sensitive to the signal. The Hedgehog signal range is probably only a few rows of cells wide; responding cells become a linear source of a long-range morphogen, that diffuses outward in all directions. There are three known Hedgehogs, Sonic (SHH), Indian (IHH) and Desert (DHH). The proteins they encode can substitute each for each other, but in wildtype animals, their distinct distributions result in unique activities. SHH controls the polarity of limb growth, directs the development of neurons in the ventral neural tube and patterns somities. IHH controls endochondral bone development and DHH is necessary for spermiogenesis. Vertebrate hedgehog genes are expressed in many other tissues, including the peripheral nervous system, brain, lung, liver, kidney, tooth primordia, genitalia and hindgut and foregut endoderm.

15

20

25

Thus, segment polarity genes have been identified in flies as mutations, which change the pattern of structures of the body segments. Mutations in these genes cause animals to develop the changed patterns on the surfaces of body segments, the changes affecting the pattern along the head to tail axis. For example, mutations in the gene *patched* cause each body segment to develop without the normal structures in the center of each segment. Instead there is a mirror image of the pattern normally found in the anterior segment. Thus, cells in the center of the segment make the

30

wrong structures, and point them in the wrong direction with reference to the overall head-to-tail polarity of the animal.

About sixteen genes in the class are known. The encoded proteins include kinases, transcription factors, a cell junction protein, two secreted proteins called wingless (WG) and the above mentioned Hedgehog (HH), a single transmembrane protein called patched (PTC) and some novel proteins not related to any known protein. All of these proteins are believed to work together in signaling pathways that inform cells about their neighbors in order to set cell fates and polarities.

PTC has been proposed as a receptor for HH protein based on genetic experiments in flies. A model for the relationship is that PTC acts through a largely unknown pathway to inactivate both its own transcription and the transcription of the *wingless* segment polarity gene. This model proposes that HH protein, secreted from adjacent cells, binds to the PTC receptor, inactivates it and thereby prevents PTC from turning off its own transcription or that of *wingless*. A number of experiments have shown coordinate events between PTC and HH.

Human *patched* gene (PTCH) was recently identified as the gene responsible for the nevoid basal cell carcinoma syndrome (NBCCS), also known as the Gorlin Syndrome, which is an autosomal dominant disorder that predisposes to both cancer and developmental defects (Gorlin (1995) *Dermatologic Clinics* 13:113-125) characterized by multiple basal cell carcinomas (BCCs), medulloblastomas and ovarian fibromas as well as numerous developmental anomalies (Hahn, H., Wicking, C., Zaphiropoulos, P.G., Gailani, M.R., Shanley, S., Chidambaram, A., Vorechovsky, I., Holmberg, E., Undén, A.B., Gillies, S., Negus, K., Smyth, I., Pressman, C., Lefell, D.J., Gerrard, B., Goldstein, A.M., Dean, M., Toftgård, R., Chenevix-Trench, G., Wainright, B. and Bale, A.E. (1996): "Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome", *Cell* 85, 841-851; and Johnson, R.L., Rothman, A.L., Xie, J., Goodrich, L.V., Bare, J.W., Bonifas, J.M., Quinn, A.G., Myers, R.M., Cox, D.R., Epstein, E.H. Jr and Scott, M.P.

(1996): "Human homolog of patched, a candidate gene for the basal cell nevus syndrome", *Science* **272**, 1668-1671). PTCH codes for a membrane receptor of the autolytically cleaved (protein spliced), amino terminal domain of *sonic hedgehog* (SHH) (Mariago, V., Davey, R.A., Zuo, Y., Cunningham, J.M. and Tabin, C.J.

5 (1996): "Biochemical evidence that patched is the Hedgehog receptor", *Nature* **384**, 176-179; and Stone, D.M., Hynes, M., Armanini, M., Swanson, T.A., Gu, Q., Johnson, R.L., Scott, M.P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper,

J.E., de Sauvage, F. and Rosenthal, A. (1996): "The tumor-suppressor gene patched

encodes a candidate receptor for Sonic hedgehog", *Nature* **384**, 129-134). In the non-signalling state, PTCH is thought to inhibit the consecutive signalling of another membrane protein, *smoothed* (SMO), however binding of SHH to PTCH relieves this inhibition (Goodrich, L.V., Milenkovic, L., Higgins, K.M. and Scott,

10 M.P. (1997): "Altered neural cell fates and medulloblastom in mouse patched mutants", *Science* **277**, 1109-1113). This cascade of signalling events, best characterized in *Drosophila*, also involves a number of intracellular components including *fused* (a serine threonine kinase), *suppressor of fused*, *costal 2*, and *cubitus interruptus* (Ruiz i Altaba, A.,: "Catching a Gli-mpse of Hedgehog" (1997) *Cell* **90**, 193-196). The latter is a transcription factor that positively regulates the expression of target genes which also include PTCH itself.

20 Mutations in the PTCH gene have been identified in both sporadic and familial BCCs (Gailani, M.R., Stähle-Bäckdahl, M., Leffell, D.J., Glynn, M., Zaphiropoulos, P.G., Pressman, C., Undén, A.B., Dean, M., Brash, D. E., Bale, A.E. and Toftgård, R. (1996): "The role of human homologue of *Drosophila* patched in sporadic basal cell carcinomas" *Nature Genet.* **14**, 78-81). The lack of the normal PTCH protein in these cells allows the constitutive signalling of SMO to occur, resulting in

25 the accumulation of mutant PTCH mRNAs (Undén, B. A., Zaphiropoulos, P.G., Bruce, K., Toftgård, R., and Stähle-Bäckdahl, M. (1997): "Human patched (PTCH) mRNA is overexpressed consistently in tumor cells of both familial and sporadic basal cell carcinoma", *Cancer Res.* **57**, 2336-2340).

WO 96/11260 discloses the isolation of *patched* genes and the use of the PTC protein to identify ligands, other than the established ligand Hedgehog, that bind thereto.

5

However, there is still a need of a further understanding of the SHH/PTCH cell signalling, which may be provided by disclosure of further genes, peptides and proteins involved therein.

10 Summary of the invention

The present invention provides a significant step forward regarding the understanding of the above described pathway. By a combination of cDNA library and RACE analysis a novel human *patched*-like gene (PTCH2) has been cloned and sequenced. Several alternatively spliced mRNA forms of PTCH2 have been identified, including transcripts lacking segments thought to be involved in sonic hedgehog (SHH) binding and mRNAs with differentially defined 3' terminal exons. Accordingly, the invention relates to isolated such mRNAs as well as to cDNAs complementary thereto.

20 Brief description of the drawings

Figure 1 shows SEQ ID NO 1, which is the amino acid sequence encoded by the novel human *patched 2* gene.

25 Figure 2 shows SEQ ID NO 2, which is the nucleotide sequence encoding the protein disclosed by SEQ ID NO 1.

Figure 3 shows SEQ ID NO 3, wherein exons and introns are designated in the genomic sequence of the novel human *patched 2* gene.

30 Figure 4A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences.

Figure 4B is a representation of the alternative splicing events that result in different C-termini.

5 Figure 4C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences.

10 ~~Figure 5A is a dark-field photomicrograph of a BCC tumor hybridized with 35 S-~~
labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells.

Figure 5B discloses PTCH2 mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.

15 Figure 5C is another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), whereas no signal is detected in epidermis (Ep).

20 Figure 5D are sections of the same tumor (C) hybridized with the PTCH2 sense probe showed no signal.

Figure 5E shows immunoreactivity for Ki-67.

25 Figure 5F discloses how tumor nests under high power magnification demonstrate abundant PATCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow).

Definitions

30 The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a

corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

5 The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state.

10 ~~The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer~~ in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

15 A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P , fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as sommonly used in a ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or monoclonal antibodies are available (*e.g.*, the peptide of SEQ ID NO 1 can be made detectable, *e.g.*, by incorporating a radio-label into the peptide, and used to detect antibodies specifically reactive with the peptide).

20 As used herein a "nucleic acid probe" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (*i.e.* A, G, C, or T) or modified bases (7-deazaguanosine, inosine, *etc.*) In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it
25 ~~does not interfere with hybridization.~~ Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages. It will be understood by one of skill in the art that probes
30 may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridization conditions. The probes

are preferably directly labeled as with isotopes, chromophores, lumiphore, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the selct sequence or subsequence.

5

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

10

The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which a nucelic acid probe is designed to specifically hybridize. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific subsequence of a larger nucleic acid to which the probe is directed or to the ovarall sequence (e.g., gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

20

The term "recombinant" when used with reference to a cell, or nucleic acid, or vector, indicates that the cell, or nucleic acid, or vector, has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid, or that the cell is derived from a cell so modified.

25

The term "identical" in the context of two nucleic acids or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorith of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson

30

and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, GESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI) or by inspection. The BLAST algorithm performs a statistical analysis of the similarity between two sequences; *see e.g.*, Karlin and Altschul (1993) *Proc. Nat'l Acad. Sci. USA* 90: 5873-5787.

The term "substantial identity" or "substantial similarity" in the context of a polypeptide indicates that a polypeptides comprises a sequence with at least 70% sequence identity to a reference sequence, or preferably 80%, or more preferably 85% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. An indication that two polypeptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

An indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

The phrase "hybridizing specifically to", refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular)

DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point TM for

the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at T_m , 50% of the probes are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, *e.g.*, an enzyme, toxin, hormone, growth factor, drug, *etc.*; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

The term "immunoassay" is an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The phrases "specifically binds to a protein" or "specifically immunoreactive with", when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins

and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory*

Manual, Cold Spring Harbour Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

A "gene product", as used herein, refers to a nucleic acid whose presence, absence, quantity, or nucleic acid sequence is indicative of a presence, absence, quantity, or nucleic acid composition of the gene. Gene products thus include, but are not limited to, and mRNA transcript and cDNA reverse transcribed from an mRNA, and RNA transcribed from that cDNA, a DNA amplified from the cDNA, an RNA transcribed from the amplified DNA or subsequences of any of these nucleic acids. Polypeptides expressed by the gene or subsequences thereof are also gene products. The particular type of gene product will be evident from the context of the usage of the term.

Detailed description of the invention

In a first aspect, the present invention relates to an isolated human protein, or an analogue or a variant thereof, capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis, such as basal cell carcinoma. The novel protein according to the invention is encoded by a novel gene, which isolated nucleic acid is described in detail below and which is denoted *patched 2* (PTCH2) due to its similarities with *patched 1* (PTCH1). Accordingly, the protein according to the invention exhibits substantial differences in sequence and functions when compared to human PTCH1 protein. The protein according to the

invention is best characterized by its functions which when compared to human PTCH1 are similar but distinct therefrom in certain ways, more specifically disclosed below in the section "Results and discussion". The novel human PTCH2 protein according to the invention is also distinct from the previously isolated mouse

5 PTCH2. Thus, in the preferred embodiment thereof, it comprises a substantial part of the amino acid sequence disclosed in SEQ ID NO. 1 of Figure 1, even though it is to be understood that the present invention encompasses any fragment, analogue or variant thereof exhibiting the biological functions of the PTCH2 protein disclosed herein. Thus, preferably, the present protein comprises at least about 1000, more
10 preferably at least about 1040 and most preferably essentially all of the amino acids of the sequence denoted SEQ ID NO. 1 of Figure 1, such as about 1100.

The proteins according to the invention are easily prepared by someone skilled in this field by recombinant DNA techniques using the molecules disclosed below or
15 any synthetic method (*see e.g.* Barany and Merrifield, Solid-Phase Peptide synthesis, pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology*, Vol. 2: Special Methods in Peptide synthesis, Part A, Merrifield *et al.*, J. Am. Chem. Soc., 2149-2156).

The present invention also relates to the use of the peptides, polypeptides and proteins disclosed herein as lead compounds in methods aimed at finding novel substances, such as substances exhibiting equivalent or even more advantageous properties than the lead compounds as such. The invention also relates to proteomic methods wherein the present molecules are used as well as to such a use *per se*.
20

25 A second aspect of the present invention is a nucleic acid encoding a protein, an analogue or a variant thereof as defined above, that is, the protein coding region of the novel human isolated PTCH2 gene. The PTCH2 gene is 57% identical to PTCH1 and 91% identical to the published mouse *Ptch2* sequence (*see* Motoyama *et al.*, (1998), supra). Thus, preferably, the nucleic acid according to the present invention comprises at least about 3000 bases, more preferably at least about 3094 ba-
30

ses and most preferably essentially all of the sequence denoted SEQ ID NO 2 of Figure 2.

5 In a specific aspect, the present invention relates to the isolated human genomic PTCH2 nucleic acid comprising parts or all of the sequence denoted SEQ ID NO 3 of Figure 3. This aspect of the invention advantageously enables design of suitable PCR primers, which in turn enables screening for mutations of all of the coding sections thereof, *e.g.* by SSCP analysis, sequencing, or any other suitable method

10 known to someone skilled in this field. Thus, the novel human PTCH2 gene according to the invention has been localized by radiation hybrid mapping to chromosome 1p32-35 with D1S211 and WI-1404 as closest flanking markers and with an estimated localization 5.5cR from D1S443. This region is often lost by LOH in various different tumor types, such as neuroblastoma, melanoma, breast cancer, colon cancer *etc.* Accordingly, PTCH2 is a candidate for a tumor suppressor gene in this
15 region and the present invention also encompass diagnostic methods based on this new disclosure.

To this chromosomal region, three cancer predisposition syndromes have also been mapped, namely, familial melanoma CMM1, modifier locus for familial adenomatous polyposis hMom1 and Michelin Tire Baby Syndrome. PTCH2 is further a candidate
20 for the gene behind these heritary syndromes. The present molecules are therefore advantageously used in the context of these conditions, *e.g.* in therapy and/or diagnosis, such as in assays.

25 Further, the invention also relates to various PCR primers based on intronic sequences, allowing amplification of all coding sequence. Such primers are advantageously used for mutation screening.

30 Further, the present invention also relates to the any isolated nucleic acid capable of specifically hybridizing to a nucleic acid according to the invention. In addition, the invention also relates to such an isolated nucleic acid which comprises one or more

mutations compared to the genomic sequence as well as the use of the novel isolated nucleic acids, *e.g.* to identify mutations for diagnostic and/or therapeutic purposes.

Further embodiments of this aspect of the invention includes nucleic acid probes,
5 *e.g.* DNA probes, labelled nucleic acids, cDNAs, RNAs *etc.*, that is, all gene products obtainable by someone skilled in this field based on the novel isolated human PTCH2 gene.

Another aspect of the invention is a nucleic acid corresponding to any one of the
10 splicing variants disclosed in Figure 4B, a protein or polypeptide encoded thereof as well as various uses thereof.

As regards the preparation of nucleic acids according to the invention, any suitable recombinant DNA technique or synthetic method may be used. (For general laboratory
15 procedures useful in this context, *see e.g.* Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 2nd ed., Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 153, Academic Press, Inc., San Diego, CA; Current Protocols in Molecular Biology, F.M. Ausbel *et al.*, eds., Current Protocols
20 (1994)).

A further aspect of the present invention is a vector comprising a nucleic acid as defined above. Vectors are *e.g.* useful for transforming cells in vitro or in vivo to express the proteins and peptides according to the invention and may *e.g.* be plasmids,
25 viruses *etc.*

Another aspect of the invention is a recombinant cell, such as a eucaryotic, *e.g.* a mammalian cell, or a procaryotic cell, *e.g.* a bacteria, comprising a vector as defined above. Such cells may *e.g.* be used to monitor expression levels of the proteins and polypeptides according to the invention in a wide variety of contexts. For example,
30 when the effects of a drug is to be determined, the drug will be administered to the

transformed organism, tissue or cell. Accordingly, model systems including such cells are another aspect of the invention.

5 A further aspect of the invention is an antibody, such as a monoclonal or polyclonal antibody, which specifically binds to a protein or polypeptide according to the invention. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more
10 amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable haecavy chain (V_H) refer to these light and heavy chains, respectively.

15 The invention also encompasses chimeric or other antibodies that binds the present proteins or polypeptides. Further, the invention also relates to the use of the present antibodies in assays. (In this context, see e.g. *Fundamental Immunology*, Third Edition, W.E. Paul, ed., Raven Press, N.Y. 1993).

20 Further, the invention also relates to a recombinant cell expressing an antibody according to the invention.

In general, prokaryotes can be used for cloning the DNA sequences encoding a human anti-PTCH2 immunoglobulin chain. *E. coli* is one prokaryotic host particularly useful for cloning the DNA sequences of the present invention. Microbes, such as
25 yeast are also useful for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase 2, isocytochrome C, and enzymes responsible for maltose and galactose utilization.
30

Mammalian cells are a particularly preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof (*see, e.g. Winnacker, From Genes to Clones*, VCH Publishers, N.Y., 1987). A number of suitable host cell lines
5 capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. Preferably, the cells are nonhuman. Expression vectors for these cells can
include expression control sequences, such as an origin of replication, a promoter,
an enhancer (Queen *et al.* (1986) *Immunol. Rev.* 89:49), and necessary processing
10 information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like (*see, e.g., Co et al.* (1992) *J. Immunol.* 1458: 1149).

15 An additional aspect of the present invention is a kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid, a polypeptide or a protein or an antibody according to the invention. Further suitable components of such a kit are easily determined
20 by someone skilled in this field as are the conditions for the use thereof.

Further, the invention also relates to the use of a nucleic acid selected from the group consisting of SEQ ID NO. 2 and SEQ ID NO. 3 in gene therapy. For a review of gene therapy procedures, see Anderson, *Science* (1992) 256:808-813; Nabel and Felgner (1993) *TIBTECH* 11: 211-217; Mitani and Caskey (1993) *TIBTECH* 11: 162-166; Mulligan (1993) *Science* 926-932; Dillon (1993) *TIBTECH* 11: 167-175; Miller (1992) *Nature* 357: 455-460; Van Brunt (1988) *Biotechnology* 6(10): 1149-1154; Vigne (1995) *Restorative Neurology and Neuroscience* 8: 35-36; Kremer and Perricaudet (1995) *British Medical Bulletin* 51(1) 31-44; Haddada et al. (1995) in *Current Topics in Microbiology and Immunology* Doerfler and Böhm (eds) Springer-Verlag, Heidelberg Germany; and Yu et al., *Gene Therapy* (1994) 1:13-26.

Delivery of the gene or genetic material into the cell is the first critical step in gene therapy treatment of disease. A large number of delivery methods are well known to those of skill in the art. Such methods include, for example liposome-based gene delivery (Debs and Zhu (1993) WO 93/24640; Mannino and Gould-Fogerite (1988) *BioTechniques* 6(7): 682-691; Rose U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner et al. (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414), and replication-defective retroviral vectors harboring a therapeutic polynucleotide sequence as part of the retroviral genome (see, e.g., Miller et al. (1990) *Mol. Cell. Biol.* 10:4239 (1990; Kolberg (1992) *J. NIH Res.* 4:43, and Cornetta et al. *Hum. Gene Ther.* 2:215 (1991)). Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof. See, e.g., Buchscher et al. (1992) *J. Virol.* 66 (5) 2731-2739; Johann et al. (1992) *J. Virol.* 66 (5):1635-1640 (1992); Sommerfelt et al., (1990) *Virol.* 176:58-59; Wilson et al. (1989) *J. Virol.* 63:2374-2378; Miller et al., *J Virol.* 65:2220-2224 (1991); Wong-Staal et al., PCT/US94/05700, and Rosenberg and Fauci (1993) in *Fundamental Immunology, Third Edition* Paul (ed) Raven Press, Ltd., New York and the references therein, and Yu et al., *Gene Therapy* (1994) supra).

The present invention may also be used in the pharmaceutical industry. For example, it will provide information that eventually may enable cells from fetal tissue, which may be transplanted into patients suffering from *e.g.* Parkinson's disease or cancer, such as BCC. (For a brief review of methods of drug delivery, *see* Langer 5 249:1 527-1533 (1990), Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985) *etc.*)

10 Detailed description of the drawings

Figure 1 shows SEQ ID NO 1, which is the amino acid sequence encoded by the novel human patched 2 gene.

15 Figure 2 shows SEQ ID NO 2, which is the nucleotide sequence encoding the protein disclosed by SEQ ID NO 1.

Figure 3 shows SEQ ID NO 3, wherein exons and introns are designated in the genomic sequence of the novel human patched 2 gene.

20 Figure 4A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences. Vertical lines indicate identical amino acids, while dots similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5' RACE analysis.

25

Figure 4B is a representation of the alternative splicing events that result in different C-termini. In the parotid gland and the colon, the penultimate and the last exon are canonically joined together. In fetal brain however the penultimate exon with part of the 3' intron functions as the terminal exon. The intronic sequence is shown by small letters with the flanking exonic by capital letters. Above the nucleotide 30 sequence, the deduced amino acid sequence is shown, and below is the correspon-

ding sequence of the mouse *Ptch2*. The conserved intronic dinucleotides are shown by bold letters and the termination signals are indicated by asterisks. Note the absence of conservation of the position of the termination codons between the mouse and human *PTCH2* sequences. The putative polyadenylation signals are also shown in this diagram. The genomic organization was obtained by analyzing BAC clones encompassing the *PTCH2* gene.

Figure 4C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences. The canonical exons 1 and 2 are shown by boxes and the intron between them by a solid line. The GT and AG dinucleotides spanning the sequences that are used as introns in individual transcripts are indicated by small letters. G, Genomic structure, derived from sequencing segments of BAC clones encompassing the *PTCH2* gene; C, Canonical transcript; A, Transcript A (the skipped exons 9 and 10 of this product are not shown in the diagram); B, Transcript B.

Figure 5A is a dark-field photomicrograph of a BCC tumor hybridized with ^{35}S -labeled antisense probe showing abundant signal for *PTCH1* mRNA (light grains) in all BCC tumor cells.

Figure 5B discloses *PTCH2* mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.

Figure 5C is another BCC showing a strong *PTCH2* mRNA signal in the periphery of the tumor nest (Tu), whereas no signal is detected in epidermis (Ep).

Figure 5D are sections of the same tumor (C) hybridized with the *PTCH2* sense probe showed no signal.

Figure 5E shows immunoreactivity for Ki-67 (brown precipitate) seen in the periphery, in the cells that showed strong upregulation of *PTCH2* mRNA.

Figure 5F discloses tumor nests under high power magnification demonstrate abundant PATCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow). Bars (A-E), 24 μ m, and F, 6 μ m.

EXPERIMENTAL

Materials and methods

The RACE analysis was performed essentially as described before (Zaphiropoulos, P.G. and Toftgård, R. (1996): "cDNA cloning of a novel WD repeat protein mapping to the 9q22.3 chromosomal region", DNA Cell Biol. 15, 1049-1056) using the Marathon kit (Promega). The primer sequences used for RACE are available upon request.

The PTCH2, 35S-labeled RNA probes used for the in situ hybridizations, that were performed as previously described (Undén *et al.*, (1997), *supra*), corresponded to positions 218 to 437 and 838 to 920 in the PTCH2 sequence of Fig. 1A.

Results and discussion

In order to identify additional components of the PTCH/SHH cascade of signalling events, the Incyte LifeSeqTM database (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA) was searched using PTCH sequences. In addition to clones representing the PTCH cDNA, two nearly identical cDNAs were identified, from the parotid gland and the colon, that contained sequences similar to, but distinct from, the 3' end of PTCH. By 5' RACE analysis using fetal brain cDNAs additional sequence information from these transcripts (termed PTCH2) and corresponding to a full length cDNA, was obtained (Fig. 4A). PTCH2 is 57% identical to PTCH1, with a significantly variable region present between the transmembrane domains 6 and 7, and 91% identical to the recently published mouse Ptch2 sequence (Motoyama, J., Takabatake, T., Takeshima, K. and Hui, C. (1998): "Ptch2, a second mouse Patched gene is co-expressed with Sonic hedgehog", Nature Genet. 18, 104-106). In simila-

rity with the mouse gene, PTCH2 lacks the C-terminal extension present in human, mouse and chicken PTCH1 (Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A., and Scott, M.P. (1996): "Conservation of the hedgehog/patched signalling pathway from flies to mice: Induction of a mouse patched gene by Hedgehog", *Genes Dev.* **10**, 301-312, Marigo, V., Scott, M.P., Johnson, R.L., Goodrich, L.V. and Tabin, C.J. (1996): "Conservation in hedgehog signalling: Induction of a chicken patched homolog by Sonic hedgehog in the developing limb", *Development* **122**, 1225-1233). However, according to the present invention, it has been shown

that the human PTCH2 cDNA terminates 36 amino acids earlier than the mouse Ptch2 sequence. Moreover, when 3' RACE was performed from fetal brain, an alternate C-terminal region was identified. This had a high structural similarity with the mouse Ptch2 C-terminal sequence and originates from the genomic region that links the last two exons of PTCH2 (Fig. 4B). Therefore, in these alternatively spliced transcripts, the penultimate exon with a segment of the contiguous 3' intron serves as the terminal exon.

Moreover the human and mouse transcripts differed in the position of the termination signals (the human sequence is 21 amino acids longer), suggesting a non-conserved, species-specific function of this alternate C-terminal domain. The finding of two possible C-terminal regions for PTCH2 is intriguing and implies a role of this phenomenon in modulating signalling. Additional alternatively spliced transcripts were also identified by the RACE analysis (Fig. 4C). Transcript A lacks the sequence that corresponds to exons 9 and 10 of PTCH1 (preliminary comparisons of the intron-exon junctions of PTCH2 with PTCH1 indicate a similar genomic organization), with the open reading frame being retained at the exon 8 to exon 11 junction. Exons 9 and 10 code for the last part of the first extracellular loop and for transmembrane domains 2 and 3 in the putative structure of the PTCH1 protein.

Furthermore this transcript also lacks a 5' segment of the canonical exon 2, due to the use of an alternative 3' splice site present in this exon, with the open reading frame being maintained. The functional consequence of this alternative splicing is not yet known, but it is interesting to note that the extracellular loops in PTCH1 are

presumed to be involved in binding of the ligand SHH (Marigo *et al.*, (1996), *Nature* **384**, supra; Stone *et al.*, (1996), *Nature* **384**, supra) and that insertion of a neo-cassette in intron 9 of the mouse PTCH1 gene is associated with a severe phenotype (Hahn, H., Wojnowski, L., Zimmer, A.M., Hall, J., Miller, G. and Zimmer, A. (1998): "Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome", *Nature Med.* **4**, 619-622). Furthermore, exons 9 and 10 encode part of a putative sterol sensing domain (Osborne, T.F. and Rosenfeld, J.M. (1998): ~~"Related membrane domains in proteins of sterol sensing and cell signalling provide~~ a glimpse of treasures still buried within the dynamic realm of intracellular metabolic regulation", *Curr. Opin. Lipidol.* **9**, 137-140, also found in PTCH1, and which has recently been implicated in mediating the potent modulating effect of cholesterol on SHH/PTCH signalling (Cooper, M.K., Porter, J.A., Young, K.E., and Beachy, P.A. (1998): "Teratogen-mediated inhibition of target tissue response to Shh signalling", *Science* **280**, 1603-1607). Thus, if PTCH2 also serves as a receptor for SHH and/or related factors, the receptor form lacking exons 9 and 10 may show altered signalling properties. Transcript B contains additional sequences between canonical exons 1 and 2, that originate from the 5' end of intron 1. The open reading frame that includes the initiator methionine of exon 1 is not maintained in this transcript, suggesting that, if this transcript is functional, either the methionine in exon 2 or non-methionine codons are used in order to produce a protein product, in similarity to what has been proposed for the alternative spliced products of human PTCH1 (Hahn *et al.*, *Cell* **85**, supra). By radiation hybrid mapping the PTCH2 gene was localized to the short arm of chromosome 1, in difference to PTCH1 residing on chromosome 9q22.3.

The mouse and zebrafish homologs of PTCH2 have been reported to be expressed in a partly overlapping pattern with PTCH1 during embryonic development and to be induced by SHH (Motoyama *et al.*, (1998) *Nature Genet.* **18**, supra, Concordet, J.P., Lewis, K.E., Moore, J.W., Goodrich, L.V., Johnson, R.L., Scott, M.P., and Ingham, P.W. (1996): "Spatial regulation of a zebrafish patched homologue reflects

the roles of sonic hedgehog and protein kinase A in a neural tube and somite patterning", Development 122, 2835-2846), implicating a role in this signalling pathway.

We were with this background interested to analyze the expression of PTCH2 in BCCs which show consistent upregulation of PTCH1 in all tumor cells (Undén *et al.*, (1997) Cancer res. 57, supra). In situ hybridization was performed on six famili-

5 *al.*, (1997) Cancer res. 57, supra). In situ hybridization was performed on six famili-
al and four sporadic BCCs of different histological subtypes. A strong positive sig-
nal for PTCH2 mRNA was observed exclusively in the tumor cells of all BCCs.
Notably, the signal was consistently stronger in the palisading peripheral cells of the
tumor nests (Fig. 4). These cells also showed a positive immunostaining for the cell
proliferation marker, Ki-67.

10 The finding that in BCCs having frequent mutations in the PTCH1 gene, the expres-
sion of the PTCH2 mRNAs is upregulated, tightly links the novel PTCH2 according
to the invention with the PTCH/SHH cascade of signalling events. It is therefore li-
kely that PTCH2 represents a target gene of this pathway which is under the negati-
ve regulation of PTCH1, precisely as PTCH1 itself. Moreover this observation
strongly suggests that PTCH2 has functions distinct from PTCH1 since upregulation
of PTCH2 expression appears unable to compensate for inactive PTCH1 protein.

15 This conclusion is also supported by the early embryonic lethality seen in PTCH1
(-/-) mice 5,13) and the lack of genetic heterogeneity in Gorlin syndrome. However,
whether PTCH2 may block the constitutive signalling of SMO, or could act as an
additional SHH receptor, possible dependent on alternative splicing, remains as the
subject of further experimentation.

CLAIMS

1. An isolated human protein or an analogue or variant thereof capable of participating in the human PTCH/SHH pathway (during embryonic development and/or carcinogenesis) comprising at least about 1040 amino acids of the sequence denoted
5 SEQ ID NO. 1 of Figure 1.

2. A protein according to claim 1, which is essentially comprised of the sequence
denoted denoted SEQ ID NO. 1 of Figure 1.

3. A nucleic acid encoding a protein according to any one of claims 1 and 2.

4. A nucleic acid encoding a protein according to claim 3 comprising at least about
3094 bases of the sequence denoted SEQ ID NO 2 of Figure 2.

5. An isolated genomic nucleic acid comprising parts or all of the sequence denoted
SEQ ID NO 3 of Figure 3.

6. An isolated nucleic acid which comprises one or more mutations compared to the
nucleic acid according to claim 5.

7. A nucleic acid having the sequence of any one of the splicing variants defined in
Figure 4B.

8. An isolated nucleic acid capable of specifically hybridizing to a nucleic acid according to any one of claims 3-6.

9. A protein or polypeptide encoded by a nucleic acid according to claim 7 or 8.

10. A vector comprising a nucleic acid according to any one of claim 3-8.

11. A recombinant cell comprising a vector according to claim 10.

12. An antibody which specifically binds to a protein according to claim 1, 2 or 9.

13. A recombinant cell expressing an antibody according to claim 12.

14. ~~A kit for the detection of a human PTCH2 gene or polypeptide comprising in a~~
container a molecule selected from the group consisting of a nucleic acid according
to any one of claims 3-8, a polypeptide or protein according to claim 1, 2 or 9 or an
antibody according to claim 12.

15. Use of a nucleic acid selected from the group consisting of SEQ ID NO. 2 and
SEQ ID NO. 3 in gene therapy.

ABSTRACT

The present invention relates to a novel human patched-like gene (PTCH2), which for the first time has been cloned and sequenced. Several alternatively spliced mRNA forms of PTCH2 have been identified, including transcripts lacking segments thought to be involved in sonic hedgehog (SHH) binding and mRNAs with differentially defined 3' terminal exons. Further, the invention also relates to the protein encoded by the present PTCH2 as well as to functional analogues and variants thereof.

Patched 2 amino acid sequence SEQ ID NO 1

1 MTRSPPLREL PPSYTTPPART AAPQILAGSL KAPLWLRAYF QGLLFSLGCG
51 IQRHCGKVL F LGLLAFGALA LGLRMAIIET NLEQLWVEVG SRVSQELHYT
101 KEKLGEEAAY TSQMLIQTAR QEGENILTPE ALGLHLQAAL TASKVQVSLY
151 GKSWDLNKIC YKSGVPLIEN GMIERMIEKL FPCVILTPLD CFWEGAKLQG
201 GSAYLPGRPD IQWTNLDPEQ LLEELGPFAS LEGFRELLDK AQVGQAYVGR
251 PCLHPDDLHC PPSAPNHHSR QAPNVAHEL S GGCHGF SHKF MHWQEELLG
301 GMARDPQGEL LRAEALQSTF LLMSPRQLYE HFRGDYQTHD IGWSEEQAST

351 VLQAWQRRFV QLAQEALPEN ASQQIHAFSS TTLDDILHAF SEVSAARVVG
401 GYLLMLAYAC VTMLRWDCAQ SQGSVGLAGV LLVALAVASG LGLCALLGIT
451 FNAATTQVLP FLALGIGVDD VFLLAHAFTE ALPGTPLQER MGECLQRTGT
501 SVVLTSINNM AAFLMAALVP IPALRAFSLQ AAIVVGCTFV AVMLVFPAIL
551 SLDLRRRH CQ RLDVLC CFSS PCSAQVIQIL PQELGDGTVP VGIAHLTATV
601 QAFTHCEASS QHVVTILPPQ AHLVPPPSDP LGSELFSPGG STRDLLGQEE
651 ETRQKAACKS LPCARWNLAH FARYQFAPLL LQSHAKAIVL VLFGALLGLS
701 LYGATLVQDG LALTDVVPRG TKEHAFLSAQ LRYFSLYEVA LVTQGGFDYA
751 HSQRALFDLH QRFSSLKAVL PPPATQAPRT WLHYRNWLQ GIQA AFDQDW
801 ASGRITRHSY RNGSEDGALA YKLLIQTGDA QELLD FSQLT TRKLVDREGL
851 IPPELFYMGL TVWVSSDPLG LAASQANFYP PPPEWLHDKY DTTGENFRIP
901 PAQPLEFAQF PFLLRGLQKT ADFVEAIEGA RAACAEAGQA GVHAYPSGSP
951 FLFEQYLGL RRCFL LAVCI LLVCTFLVCA LLLNPNW TAG LIVLVLAMMT
1001 VELFGIMGFL GIKLSAIPVV ILVASVGIGV EFTVHVALGF LTTQGSRLR
1051 AAHALEHTFA PVT DGAISTL LGLLMLAGSH FDFIVRYFFA ALTVLTLLGL
1101 LHGLVLLPVL LSILGPPPEV IQMYKESPEI LSPPAPQGGG LRPEEI

Fig. 1

1 **ATG**ACTCGAT CGCCGCCCCCT CAGAGAGCTG CCCCCGAGTT ACACACCCCC
51 AGCTCGAACC GCAGCACCCC AGATCCTAGC TGGGAGCCTG AAGGCTCCAC
101 TCTGGCTTCG TGCTTACTTC CAGGGCCTGC TCTTCTCTCT GGGATGCGGG
151 ATCCAGAGAC ATTGTGGCAA AGTGCTCTTT CTGGGACTGT TGGCCTTTGG
201 GGCCCTGGCA TTAGGTCTCC GCATGGCCAT TATTGAGACA AACTTGGAAC
251 AGCTCTGGGT AGAAGTGGGC AGCCGGGTGA GCCAGGAGCT GCATTACACC

301 ~~AAGGAGAAGC TGGGGGAGGA GGCTGCATAC ACCTCTCAGA TGCTGATACA~~
351 GACCGCACGC CAGGAGGGAG AGAACATCCT CACACCCGAA GCACTTGGCC
401 TCCACCTCCA GGCAGCCCTC ACTGCCAGTA AAGTCCAAGT ATCACTCTAT
451 GGGAAGTCCT GGGATTTGAA CAAAATCTGC TACAAGTCAG GAGTTCCCCT
501 TATTGAAAAT GGAATGATTG AGCGGATGAT TGAGAAGCTG TTTCCGTGCG
551 TGATCCTCAC CCCCCTCGAC TGCTTCTGGG AGGGAGCCAA ACTCCAAGGG
601 GGCTCCGCCT ACCTGCCCCG CCGCCCGGAT ATCCAGTGGA CCAACCTGGA
651 TCCAGAGCAG CTGCTGGAGG AGCTGGGTCC CTTTGCCCTCC CTTGAGGGCT
701 TCCGGGAGCT GCTAGACAAG GCACAGGTGG GCCAGGCCTA CGTGGGGCGG
751 CCCTGTCTGC ACCCTGATGA CCTCCACTGC CCACCTAGTG CCCCCAACCA
801 TCACAGCAGG CAGGCTCCCA ATGTGGCTCA CGAGCTGAGT GGGGGCTGCC
851 ATGGCTTCTC CCACAAATTC ATGCACTGGC AGGAGGAATT GCTGCTGGGA
901 GGCATGGCCA GAGACCCCCA AGGAGAGCTG CTGAGGGCAG AGGCCCTGCA
951 GAGCACCTTC TTGCTGATGA GTCCCCGCCA GCTGTACGAG CATTTCCGGG
1001 GTGACTATCA GACACATGAC ATTGGCTGGA GTGAGGAGCA GGCCAGCACA
1051 GTGCTACAAG CCTGGCAGCG GCGCTTTGTG CAGCTGGCCC AGGAGGCCCT
1101 GCCTGAGAAC GCTTCCCAGC AGATCCATGC CTTCTCCTCC ACCACCCTGG
1151 ATGACATCCT GCATGCGTTC TCTGAAGTCA GTGCTGCCCC TGTGGTGGGA

1201 ~~GGCTATCTGC TCATGCTGGC CTATGCCTGT GTGACCATGC TCGGGTGGGA~~

Fig. 2

1251 CTGCGCCCAG TCCCAGGGTT CCGTGGGCCT TGCCGGGGTA CTGCTGGTGG
1301 CCCTGGCGGT GGCTCAGGC CTTGGGCTCT GTGCCCTGCT CGGCATCACC
1351 TTCAATGCTG CCACTACCCA GGTGCTGCCC TTCTTGCTC TGGGAATCGG
1401 CGTGGATGAC GTATTCCTGC TGGCGCATGC CTTACAGAG GCTCTGCCTG
1451 GCACCCCTCT CCAGGAGCGC ATGGGCGAGT GTCTGCAGCG CACGGGCACC
1501 AGTGTCTGAC TCACATCCAT CAACAACATG GCCGCCTTCC TCATGGCTGC
1551 CCTCGTTCCC ATCCCTGCGC TGCAGGCCTT CTCCCTACAG GCGGCCATAG
1601 TGGTTGGCTG CACCTTTGTA GCCGTGATGC TTGTCTTCCC AGCCATCCTC

1651 AGCCTGGACC TACGGCGGCG CCACTGCCAG CGCCTTGATG TGCTCTGCTG
1701 CTTCTCCAGT CCCTGCTCTG CTCAGGTGAT TCAGATCCTG CCCCAGGAGC
1751 TGGGGGACGG GACAGTACCA GTGGGCATTG CCCACCTCAC TGCCACAGTT
1801 CAAGCCTTTA CCCACTGTGA AGCCAGCAGC CAGCATGTGG TCACCATCCT
1851 GCCTCCCCAA GCCACCTGG TGCCCCCACC TTCTGACCCA CTGGGCTCTG
1901 AGCTCTTCAG CCCTGGAGGG TCCACACGGG ACCTTCTAGG CCAGGAGGAG
1951 GAGACAAGGC AGAAGGCAGC CTGCAAGTCC CTGCCCTGTG CCCGCTGGAA
2001 TCTTGCCCAT TTCGCCCCT ATCAGTTTGC CCCGTTGCTG CTCCAGTCAC
2051 ATGCTAAGGC CATCGTGCTG GTGCTCTTTG GTGCTCTTCT GGGCCTGAGC
2101 CTCTACGGAG CCACCTTGGT GCAAGACGGC CTGGCCCTGA CGGATGTGGT
2151 GCCTCGGGGC ACCAAGGAGC ATGCCTTCCT GAGCGCCCAG CTCAGGTACT
2201 TCTCCCTGTA CGAGGTGGCC CTGGTGACCC AGGGTGGCTT TGA CTAGGCC
2251 CACTCCCAAC GCGCCCTCTT TGATCTGCAC CAGCGCTTCA GTTCCCTCAA
2301 GGCGGTGCTG CCCCCACCGG CCACCCAGGC ACCCCGCACC TGGCTGCACT
2351 ATTACGCAA CTGGCTACAG GGAATCCAGG CTGCCTTTGA CCAGGACTGG
2401 GCTTCTGGGC GCATCACCCG CCACTCGTAC CGCAATGGCT CTGAGGATGG
2451 GGCCCTGGCC TACAAGCTGC TCATCCAGAC TGGAGACGCC CAGGAGCTTC
2501 TGGATTTCAG CCAGCTGACC ACAAGGAAGC TGGTGGACAG AGAGGGACTG

2551 ATTCCACCCG AGCTCTTCTA CATGGGGCTG ACCGTGTGGG TGAGCAGTGA

Fig. 2 (forts)

2601 CCCCCTGGGT CTGGCAGCCT CACAGGCCAA CTTCTACCCC CCACCTCCTG
2651 AATGGCTGCA CGACAAATAC GACACCACGG GGGAGAACTT TCGCATCCCG
2701 CCAGCTCAGC CCTTGGAGTT TGCCCAGTTC CCCTTCCTGC TCGTGGCCT
2751 CCAGAAGACT GCAGACTTTG TGGAGGCCAT CGAGGGGGCC CGGGCAGCAT
2801 GCGCAGAGGC CGGCCAGGCT GGGGTGCACG CCTACCCCAG CGGCTCCCCC
2851 TTCCTCTTCT GGGAACAGTA TCTGGGCCTG CGGCGCTGCT TCCTGCTGGC
2901 CGTCTGCATC CTGCTGGTGT GCACTTTCCT CGTCTGTGCT CTGCTGCTCC
2951 TCAACCCCTG GACGGCTGGC CTCATAGTGC TGGTCCTGGC GATGATGACA

3001 GTGGAAGTCT TTGGTATCAT GGGTTTCCTG GGCATCAAGC TGAGTGCCAT
3051 CCCCCTGGTG ATCCTTGTGG CCTCTGTAGG CATTGGCGTT GAGTTCACAG
3101 TCCACGTGGC TCTGGGCTTC CTGACCACCC AGGGCAGCCG GAACCTGCGG
3151 GCCGCCCATG CCCTTGAGCA CACATTTGCC CCCGTGACCG ATGGGGCCAT
3201 CTCCACATTG CTGGGTCTGC TCATGCTTGC TGGTTCCCAC TTTGACTTCA
3251 TTGTAAGGTA CTTCTTTGCG GCGCTGACAG TGCTCACGCT CCTGGGCCTC
3301 CTCCATGGAC TCGTGCTGCT GCCTGTGCTG CTGTCCATCC TGGGCCCCGCC
3351 GCCAGAGGTG ATACAGATGT ACAAGGAAAG CCCAGAGATC CTGAGTCCAC
3401 CAGCTCCACA GGGAGGCGGG CTTAGGCCCG AGGAGATC**T A G**

Fig. 2 (forts)

Patched 2 Intron-Exon organization SEQ ID NO 3

The intron sequences between exons 2 - 3 and exons 18 - 19 are missing (introns: small letters, exons: capital letters). Small letters in the first exon indicate nucleotides that have not been unambiguously determined.

The start and termination codons are indicated.

Exon 1

```

1  CGGGTGAATC CCGGCGCCGC GCCCGGACC CGCAGCTCCC TGCACTCCTC
51  CCTCCCAGCC GCTTTAACAC CCACACCCCA CAGTCTCTCC CACGsCCGCG
101 CCTTGCGCGC CCCACTGAAT CCCTACGCGG GGCCCAGCGG TACCGGGAGA
151 CCGGGCTAGC CTATGGGAGC GCCCAGATAA CGCGGGTTGG GGGCGCCCGC
201 GCCCcCATCC CCGCCAGCAT GACTCGATCG CCGCCCCTCA GAGAGCTGCC
251 CCCGAGTTAC ACACCCCCAG CTCGAACCGC AGCACCCCAG gtgagtagag
301 ggggagctgg aagaaggaag agagcggagc caggtctgtc actcgggcct
351 ctgcaagggtt tgtgatgtct tgaagtgccg agtgtcatta gatgtctgaa
401 ggcaagtgag agccagcacc gcaagcaagt tgtgcgtgtg tgtcgggtgtg
451 tctgtgccgg tgtctcctca tcgtctggcc agtgagaatg aatgtctgtg
501 ggttcacctc tgtgtccacc cgacgacagg tgtgtgtaca tatgtatcct
551 gctctcagaa aatgggccta tgccgccggg cgcggtgact cacgcctgta
601 atcccaacac tgggaggctg aggcaggcag attacctgag gtcaggagtt
651 cgagaccagc caggccaaca tggggaaact ctgtctctac taaaaataaa
701 aattagcagg gcgtggtggc gggcgccctgt agtcccaact actcgggagg
751 ctgaggcagg agaatctctt gaacctggga ggcggagggtt gcagtcaagc
801 cgagatcaca cactgcact ccagccaggg caacagagcg agatgcgtct
851 caaaaaaaaa aaaaaaaaaa aaaaggagag aaaacaaaaa gaaaagaaag
901 gaaaataggc ctatgccttc ctcagggtgtg tgctggggat ggtgggtgtt
951 acatcttcca agtctgggcc tgtgtctgtg ttggtgctcc ctgtcccaca
1001 tccagaaatc aagaagcgag ggctgggcag cagatataca gggtgagaag

```

Fig. 3

1051 ggaaggattt catgcattgt tacagtgatg cctggctgac ccttctcttt
 EXON 2
 1101 ccatcccaga TCCTAGCTGG GAGCCTGAAG GCTCCACTCT GGCTTCGTGC
 1151 TTA CTTCCAG GGCCTGCTCT TCTCTCTGGG ATGCGGGATC CAGAGACATT
 1201 GTGGCAAAGT GCTCTTTCTG GGA CTTGTTGG CCTTTGGGGC CCTGGCATT
 1251 GGTCTCCGCA TGGCCATTAT TGAGACAAAC TTGGAACAGC TCTGGGTAGA
 1301 AGTGGGCAGC CGGGTGAGCC AGGAGCTGCA TTACACCAAG GAGAAGCTGG
 1351 GGGAGGAGGC TGCATACACC TCTCAGATGC TGATACAGAC CGCACGCCAG
 1401 GAGGGAGAGA ACATCCTCAC ACCCGAAGCA CTTGGCCTCC ACCTCCAGGC

 1451 AGCCCTCACT GCCAGTAAAG TCCAAGTATC ACTCTATGGG AAG.....
 1501g
 1551 tgagtctggc tgagccctg agcagctggg ggcgaggcgt gctgtggggg
 1601 ttctggagtg ggaatcccct tcttctgctg atctcctatg cccctggcta
 EXON 4
 1651 ttgcagTCCT GGGATTTGAA CAAAATCTGC TACAAGTCAG GAGTTCCCT
 1701 TATTGAAAAT GGAATGATTG AGCGGgtaag tgtcctgaga gggagtagag
 1751 gcagaacttt ttctgtagcg tgggaggact cagagaccga gcaagcccca
 1801 cagcctgcaa tctgccccct taaaactaag gagggggatt gcagagggca
 1851 tcctacaaag gttgtggggc aggactgacg tggcccgggg tatccctggc
 EXON 5
 1901 agATGATTGA GAAGCTGTTT CCGTGCGTGA TCCTCACCCC CCTCGACTGC
 1951 TTCTGGGAGG GAGCCAAACT CCAAGGGGGC TCCGCCTACC TGCCgtgagt
 2001 gccactcctg gggccctgct tcatctcccg ctggggactc tcccagcaga
 2051 aaggaggggt ctggggaatg aggatgatca aaaccttacc aaggtcctaa
 2101 ttacctccca ggccaggaac agagagcatg ggcttcccca aggctctctc
 2151 cacatcctcc ttctctttcc ctctcaagga aggaagacct gacttattta
 2201 cacaaaacta aacacaaaga tctgtaagat ctgagcaaag gagaaaaaga
 2251 tccccacaaa gaggctttgc tgggggaaat tacctaggtg tttgctaagc
 2301 cattgcccag gccagaaaga aaacctgcta caggcatgtg cctgctggtt

 2351 gtatattaga accaagcaca cagcttggtg aggaactcag tggggccttt

Fig 3 (forts)

2401 ctgggccctt tctatgtatt aggtaaccct gccctgatat tcgtctcagc
 2451 cccttggtact cttctacagc tcaactgtagc accctgggtgg gcccatgcag
 2501 cctggcagtt ctgagaagct gaggcttgca caccctccat atggaaggac
 2551 aaatcggcag ataagaggag ggtgggggtac agcatggcgc cccagcagca
 2601 gtttggagcc tgggttttcg tccctgacct tcaccaacta taggcttttc
 2651 cctcagCGGC CGCCCGGATA TCCAGTGGAC CAACCTGGAT CCAGAGCAGC
 2701 TGCTGGAGGA GCTGGGTCCC TTTGCCTCCC TTGAGGGCTT CCGGGAGCTG
 2751 CTAGACAAGG CACAGGTGGG CCAGGCCTAC GTGGGGCGGC CCTGTCTGCA

 2801 CCCTGATGAC CTCCACTGCC CACCTAGTGC CCCCACCAT CACAGCAGGC
 2851 AGgtgggttc caaccaggtc tgccaggga aggctgtttt ccttcctttt
 2901 cccttcctca tactcctgtg ttctggggga gctgactgct ctgtgccctg
 2951 accccccact tcctggccat tattaccctg ctcccacagt gccaggcccc
 3001 caatgttcca ttcccattca gttatcctac ggagccctca agtgggtatat
 3051 atgaatccct ttttcctttt ctaagcctag ataaggctgg acttcctttt
 3101 tttttttttt ttgagtctca ctctgtcacc caggctggag tgcagtagtt
 3151 cgatcttggc tcaactgcaac ctcggtcaa gcaattctcc tgccttagcc
 3201 tcctgagtag ctgggattac aggtgcccac caccatgccc ggctaatttt
 3251 tattagcctc ccaaagtgtt gggattacag gcgtgagcca ctgcgcctgg
 3301 ccaaggctgg actttttatc aaaatagact aatacaggga aactaagaac
 3351 acagcaggtg agcatgaata tcatacctgg tttcccagggt ttctttgtgg
 3401 ccctgcaaat gtggtacttt tttcagaatc cgccagttac accagctcct
 3451 cccagaagcc tacttccagg cctctgcttc cccttggggc ttctgtctg
 3501 cgggatacta gctgttcact cctgcagagc agtcaagagg ctccagaatag
 3551 ttacctacac tccagcccta ctgagcttca tggcagcgtg gttcctggag
 3601 gtggaagccc agggacactc agttatccac ggccagggcc ttgagcatta
 3651 acccctcctg ttcccctcca gGGCTCCCAA TGTGGCTCAC GAGCTGAGTG

 3701 GGGGCTGCCA TGGCTTCTCC CACAAATTCA TGCACTGGCA GGAGGAATTG

Fig 3 (forts)

[illegible]

5101 CGGGCACCAG TGTCGTACTC ACATCCATCA ACAACATGGC CGCCTTCCTC
 5151 ATGGCTGCCC TCGTTCCCAT CCCTGCGCTG CGAGCCTTCT CCCTACAGGC
 5201 GGCCATAGTG GTTGGCTGCA CCTTTGTAGC CGTGATGCTT GTCTTCCCAG
 5251 CCATCCTCAG CCTGGACCTA CGGCGGCGCC ACTGCCAGCG CCTTGATGTG
 5301 CTCTGCTGCT TCTCCAGgta ctgcgtgcgc cccagcccct tcctcccgtg
 5351 acccacgcca gcctgtcccc tcaccagcat ttcaaggcac agacctgtca
 5401 tccactctct acctcttcca gTCCCTGCTC TGCTCAGGTG ATTCAGATCC
 5451 TGCCCCAGGA GCTGGGGGAC GGGACAGTAC CAGTGGGCAT TGCCCCACCTC

 5501 ACTGCCACAG TTCAAGCCTT TACCCACTGT GAAGCCAGCA GCCAGCATGT
 5551 GGTCACCATC CTGCCTCCCC AAGCCCACCT GGTGCCCCCA CCTTCTGACC
 5601 CACTGGGCTC TGAGCTCTTC AGCCCTGGAG GGTCCACACG GGACCTTCTA
 5651 GGCCAGGAGG AGGAGACAAG GCAGAAGGCA GCCTGCAAGT CCCTGCCCTG
 5701 TGCCCGCTGG AATCTTGCCC ATTTGCCCCG CTATCAGTTT GCCCCGTTGC
 5751 TGCTCCAGTC ACATGCTAAG gtaagactgg gcagagcagg gcagagactt
 5801 agcatctctg ggcccagaag ggcagagagg gcttagtcca ctgcctgagg
 5851 ggctgggggc agccctgggg tctccagctt agttgctaca tcccgcagGC
 5901 CATCGTGCTG GTGCTCTTTG GTGCTCTTCT GGGCCTGAGC CTCTACGGAG
 5951 CCACCTTGGT GCAAGACGGC CTGGCCCTGA CGGATGTGGT GCCTCGGGGC
 6001 ACCAAGGAGC ATGCCTTCCT GAGCGCCCAG CTCAGGTACT TCTCCCTGTA
 6051 CGAGGTGGCC CTGGTGACCC AGGGTGGCTT TGA CTACGCC CACTCCCAAC
 6101 GCGCCCTCTT TGATCTGCAC CAGCGCTTCA GTTCCCTCAA GGCGGTGCTG
 6151 CCCCCACCGG CCACCCAGGC ACCCCGCACC TGGCTGCACT ATTACCGCAA
 6201 CTGGCTACAG Ggtgagaggc gaggagacgg gcagggaggg gtgctgcagg
 6251 gagaaacgcc ctgggggccac cagctaataag aaccctatcc tgggtctcccc
 6301 cagGAATCCA GGCTGCCTTT GACCAGGACT GGGCTTCTGG GCGCATCACC
 6351 CGCCACTCGA CCGCAATGGC TCTGAGGATG GGGCCCTGGC CTACAAGCTG

 6401 CTCATCCAGA CTGGAGACGC CCAGGAGCTT CTGGATTTC A GCCAGgttgg

Fig 3 (forts)

100

7801 aagggacaga gccctgtggc ccacagacag gtacctcccc aacagggtgcc
 7851 accagctgaa ggtggcagcc tcctcctttc cccagacacc atgttcctgc
 7901 ccctcagccc tcctggcttc ttcattgggac ccaccttaga ctttttaggat
 7951 ccagaacaag gtgcaggggt tgccccaggc ctcaacatcc tgtcgcctgc
 8001 cagctctcat atcctgctgg agaccaacaa gggccccagc ttcccaacag
 8051 tcatggtaat ccccagcgag atgctaaagg ggacgggagc cccagggggc
 8101 cgtgggctta ctggggctgg tgtctcccca cagGTGATAC AGATGTACAA
 8151 GGAAAGCCCA GAGATCCTGA GTCCACCAGC TCCACAGGGA GGCGGGCTTA

 8201 Ggtggggggc atcctcctcc ctgccccaga gctttgccag agtgactacc
 8251 tccatgaccg tggccatcca cccaccccc ctgcctggtg cctacatcca
 8301 tccagcccct gatgagcccc cttgggtccc tgctgtcact agctctggca
 8351 acctcagttc caggggacca ggtccagcca ctgggtgaaa gagcagctga
 8401 agcacagaga ccatgtgtgg ggcgtgtggg gtcactggga agcactgggt
 8451 ctggtgttag acgcaggatg gacccctgga gggctctgct gctgctgcat
 8501 cccctctccc gaccagctg tcatgggcct ccctgatatc catacagaac
 8551 agccaccgat ttgcacatcc aggcctgtgt gagcctgtat ctgtgtcact
 8601 tgagagtgaag agctggcact tggggctgca gtgcagccct gtcccccttc
 8651 ccaccccaca ccaactgcctg cccagctgac caagcctgag ggaccctcca
 8701 gcaccccttc gtctggtgac tcctgggcag gctctccata tcctgcccc
 8751 cctcctacca catccattat ttatatgaaa atgtctattt ttgtagtata
 8801 catacatgtt agctatgatg aaagttttat tttttaaaga atgaaatata
 8851 ttctatgtga agctatgatg aaagttttat tttttaaaga atgaaatata
 8901 ttctatgtga actaatctcg aaagttttat tttttaaaga atgaaatata
 8951 ttctatgtgt gcaagtgaac attagcttca gttgcttttt ttggacaga
 9001 gtggggagtt tgcaagtga ctagctat tggaaggagc ttctctgggtg
 9051 ccaggacctg aggtattagc ttctctagtt ctgggtggaa aagaccccag

 9101 attctggatt ttgtcatat acttggtaac atcatctgga ttaagtgctt

Fig 3 (forts)

9151 actatacaaa acgataacaa attttggttg tgtgaaatcc tactgggttc
 9201 aatctggaga ccgagagcag aaaaaaaga accccactgt gtggctttca
 9251 gagccaccat attccagcct gccgtctct ccagactcac ctccacctac
 9301 ctgcttcacc cgcacgggaa acggcaaggc agaggggcaa agccatgcag
 9351 caggtggaag gcgaggtgga ggcagatcag gaaagcagcc agttgaagca
 9401 gagagaggtc aacagggctc ggggagcttc tcaggaggtt tgtggacca
 9451 gggaaaggag ccaggttcca gagcaacctc caaggcaaag gcctctgtaa
 9501 gttggttgct ctgacagccg agaggtgtct ttggccagtc agccagtgga

 9551 ~~tcagttgcgg gaactgctca gaaactgagg tgctagcagt tagtgaggac~~
 9601 acagcgtaag ttgtttgttc tgtgaaagtt gaacagctcc actaagcaga
 9651 ggccttgaag agtggccaca gccctggaat agagcacaga gcctcaccta
 9701 gaggcgtggg gaggtttgca actgcccctt cccagccata gcttaggacc
 9751 catagtctag ttcacataga ccttgggctc caaccacca ctcaccagga
 9801 atgatccac cccaggaaca atgcgttctc acatcccacc ccacctggac
 9851 aaaggccagg aaatcatgtt ctgacaaaaa gatacaacaa caaaaacaac
 9901 aacaacaaaa aacgcctatt gcaattgaat ccacgctaaa atgcctaaaa
 9951 agctcaagag aagcgggtag ttggcagaga acctagagta gggggtgcaa
 10001 ccagcaggcc caagggaggg aggctgcatt tgggtccagc agtggttggg
 10051 tcaccaagaa gggccttcta ggtggagcag agagagctca ccaggccaga
 10101 atagtgcaa gggggtcagc cctcagtgcc acttaccagc ggagtaacc^E
 10151 tgggcaagtt agccagcctc actaagcctc cccatcttca tctttccagG
 XON 22
 10201 CCCGAGGAGA TC**TAG**CCTCT GCCTCCCACC CCAGCACCCC CTCATCAGAC
 10251 ACAAGGAGCG CCACTGTCTG GACAGGCTGA ATTGGTCTTC GGGTCCCTAA
 10301 TTTCTCATAC GCCATTCCCT CTGCCTAGAA CACTTTCTCA CCTCCCCTTG
 10351 ATGTGACCCC ATATCACCTT TCGAGGTGAA TTGGATCGGA TGCCATCTCC
 10401 TCCAGGAGGG GTGGGGTCGT GCCTCCTGTG AGGTCCCAGT GCCCCTGAGT

 10451 GTCTGTGCCC GTCTGTTTCC CCGTCCCTCT CTCTAAGCCC GGAGGCTTAC

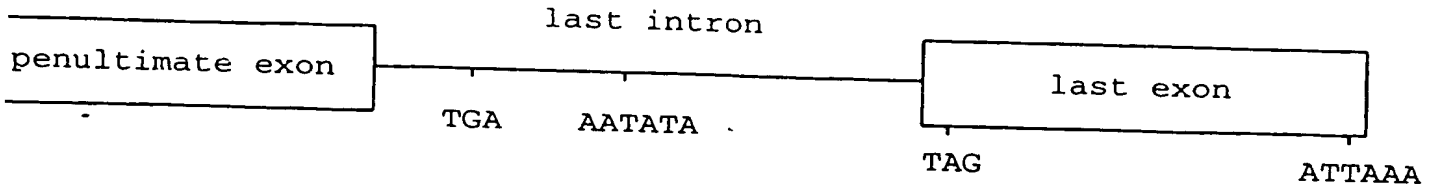
Fig 3 (forts)

10501 TCGGGGTAAG GACGGCGGGA CAGGACCTTA ACCCCTGGGA CGAACACCAG
10551 CTCCGCAAAG GACTCCGCAC CCGGCGCCGC CCACGGGGTG CGGGTCCCAG
10601 GAGGACCAGC AGAGAGGAGC ATAGGAGAGC AAAGGAGATC AGTGACCCAT
10651 GGCTTCCCCG GTGGCGCGGA ACAGCCCGGA GCCGCCTGTG ATTTGCATAC
10701 CCATGGTGCA CCACGAAAAG ATACCCTCAA GATGCTTGCA CTCCCTCTGT
10751 GCGCGCATTT CTGCACTGTT TTAGAGCATG ATGCCTCTTA CACGCATCTG
10801 TGTGCATAAA CTACATATAG GGAGTGCCTA CCACGCAGGC ATCCAACAAC
10851 CATAAGTGTG TTAAGTGTTA GTTCTCCCTG CGAGGTTCGA AGCGGAAGTC
10901 ACGAATATAC TCGGGTTTCT CTTCAAAGCG CATAAATCTT TCGCCTTTTA
10951 CTAAAGATTT CCGTGGAGAG AAAGTTGTGA GTTTTTATTG AATTTTTTTGA
11001 GGCCTCTTAT TTCCTGAGGC TACATTTTTA AGTATTAAAA GTTAGGCAAC
11051 TACAAAAAAA AAAAAAAA

Fig 3 (forts)

100

Genomic



GlyLeuArgTrpGlyAlaSerSerSerLeuProGlnSerPheAlaArgValThr
 GGGCTTACgtggggggcatcctcctcctgccccagagctttgccagagtgact
 -----C-----acaggc--c--a-----t-----

ThrSerMetThrValAlaIleHisProProProLeuProGlyAlaTyrIleHis
 acctccatgaccgtggccatccacccacccctgacctggtgcctacatccat
 -----t-----c-----a--t-----a--a--g----c

ProAlaProAspGluProProTrpSerProAlaAlaThrSerSerGlyGlnLeu
 ccagcccctgatgagcccccttggtcccctgctgccactagctctggcaacctc
 -----t-c--g-----a-a-a-
 *

SerSerArgGlyProGlyProAlaThrGly * gProAsnAsnIle *
 agttccaggggaccagggtccagccactgggtga.....agGCCCCGAGGAGATCTAG

Parotid gland
 Colon



Fetal brain

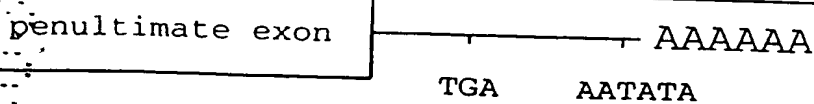
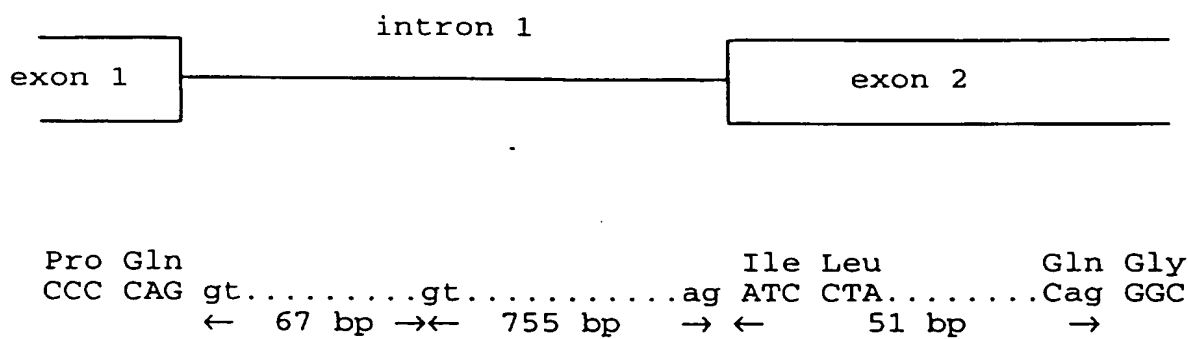
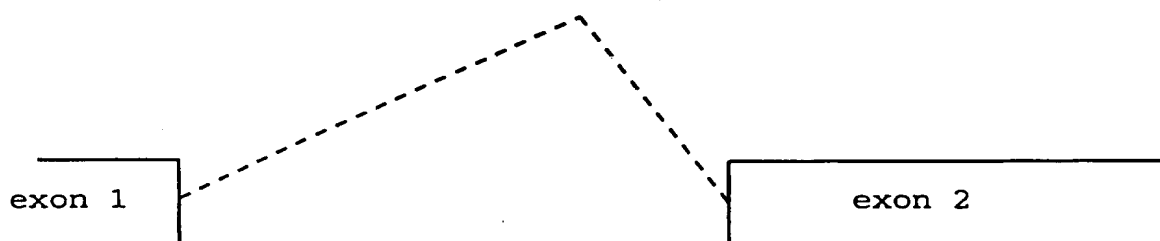


Fig. 4B

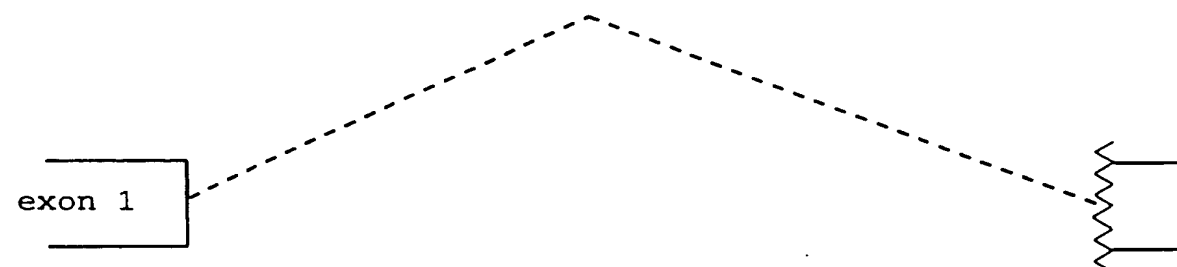
G



C



A



B

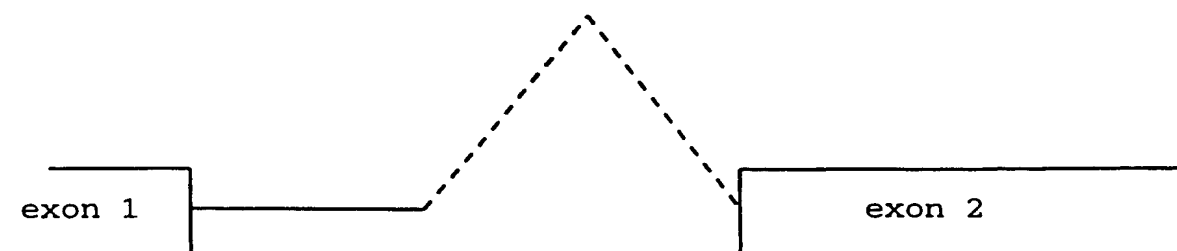


Fig. 4C

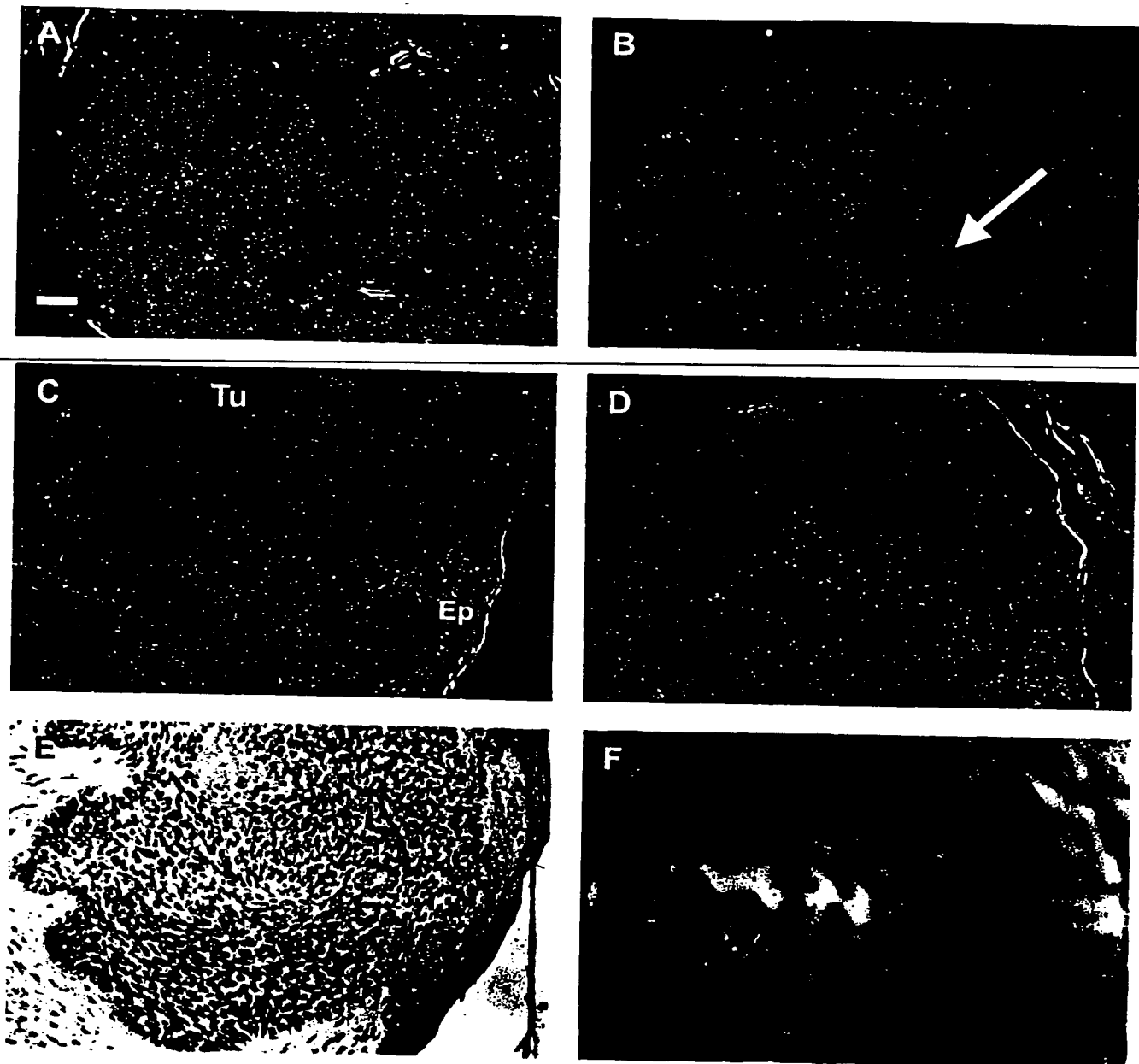


Fig. 5